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Does Zinc Moderate Essential Fatty Acid and Amphetamine Treatment of Attention-Deficit/Hyperactivity Disorder?

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Abstract

Zinc is an important co-factor for metabolism relevant to neurotransmitters, fatty acids, prostaglandins, and melatonin, and indirectly affects dopamine metabolism, believed intimately involved in attention-deficit/hyperactivity disorder (ADHD). To explore the relationship of zinc nutrition to essential fatty acid supplement and stimulant effects in treatment of ADHD, we re-analyzed data from an 18-subject double-blind, placebo-controlled crossover treatment comparison of d-amphetamine and Efamol (evening primrose oil, rich in gamma-linolenic acid). Subjects were categorized as zinc-adequate ($n = 5$), borderline zinc ($n = 5$), and zinc-deficient ($n = 8$) by hair, red cell, and urine zinc levels; for each category, placebo-active difference means were calculated on teachers' ratings. Placebo-controlled d-amphetamine response appeared linear with zinc nutrition, but the relationship of Efamol response to zinc appeared U-shaped; Efamol benefit was evident only with borderline zinc. Placebo-controlled effect size (Cohen's d) for both treatments ranged up to 1.5 for borderline zinc and dropped to 0.3-0.7 with mild zinc deficiency. If upheld by prospective research, this post-hoc exploration suggests that zinc nutrition may be important for treatment of ADHD even by pharmacotherapy, and if Efamol benefits ADHD, it likely does so by improving or compensating for borderline zinc nutrition.

Introduction

Zinc is necessary for 100 different metalloenzymes and metal-enzyme complexes (Toren et al. 1996), many of them in the central nervous system. It contributes to structure and function of brain (Black 1998). Among other things, zinc is necessary for conversion of dietary pyridoxine (vitamin B₆) to its active form, pyridoxal phosphate. Vitamin B₆ is necessary in this form for the conversion of tryptophan to serotonin. Importantly, zinc is also necessary for production and modulation of melatonin, which helps regulate dopamine function (Sandyk 1990; Chen et al. 1999), widely believed to be a key factor in attention- deficit/hyperactivity disorder (ADHD) and its treatment. In fact, Sandyk (1990) hypothesized that parasympathomimetic stimulants, at least d-amphetamine, work in ADHD partly via effects on melatonin.

Both animal data (e.g., Halas and Sandstead 1975; Sandstead et al. 1977; Golub et al. 1996) and human findings suggest involvement of zinc deficiency in hyperactivity. Studying moderately zinc-deprived monkeys, Golub et al. (1996) reported attentional impairment at levels that did not cause growth retardation. They concluded that activity and attention can be affected during early stages of zinc deprivation before growth retardation. Human zinc deficiency syndrome includes concentration impairment and jitters (Aggett and Harries 1979), and zinc deficiency can delay

cognitive development (Black 1998). In ADHD, zinc has been reported significantly ($p < 0.001$) deficient compared to controls, with effect size (E.S., Cohen's d) up to 2.4 (Bekaroglu et al. 1996; Kozielec et al. 1998; Toren et al. 1996). Starobrat-Hermelin (1998) found a high rate of magnesium, zinc, iron, copper, and calcium deficiencies in 116 children with ADHD on the basis of serum, red cell, and hair analyses. Hair zinc was lower in ADHD with comorbid oppositional-defiant or conduct disorder than in ADHD alone or with anxiety. Bekaroglu et al. (1996) concluded that "zinc deficiency may play a role in aetiopathogenesis of ADHD."

Zinc is also involved in essential fatty acid (EFA) metabolism in several ways (Fogerty et al. 1985; Bekaroglu et al. 1996; Poling et al. 1996; Prows and Schroeder 1997). It is a co-enzyme for delta-6-desat-urase (e.g., Bettger et al. 1979; Huang et al. 1982; Eder and Kirchgeßner, 1996) and modulates cyclooxygenase activity (Sakuma et al. 1996). The latter enzyme is necessary for production of cell-regulating prostaglandins and thromboxanes from EFA precursors. In turn, zinc absorption from the gut is facilitated by prostaglandins of the 2 series (Song and Adham 1980). EFAs and their metabolites are also involved in dopamine and norepinephrine metabolism (Di Marzo and Piomelli 1992; Gross et al. 1997; Molderings et al. 1992; Gomez-Nino et al. 1992; Sherbourne et al. 1992; Racke et al. 1992; Negishi and Ito 1992) and vice versa (Alanko et al. 1992; Nadasy et al. 1992; Weidenfeld 1992). Zinc may exert a protective effect against the oxidative risk of high n-3 fatty acid intake (Villet et al. 1997). Bekaroglu et al. (1996) reported a significant correlation between serum zinc and serum free fatty acid levels in 48 children with ADHD, who had significantly lower free fatty acids and zinc than 45 healthy volunteer children (E.S. > 1.6 ; $p < 0.001$).

Thus, it is reasonable to suspect a synergism of zinc and EFAs in facilitating and regulating dopamine, norepinephrine, and, possibly, serotonin activity, with implications for stimulant treatment of ADHD. Arnold et al. (1990) reported a significant correlation of baseline hair zinc with placebo-controlled d-amphetamine response in ADHD, but did not explore relationships with fatty acids or use additional tissue measures of zinc. This report explores further the relationship of zinc nutrition, essential fatty acid effects, and stimulant effects in treatment of ADHD, utilizing three measures of zinc nutritional status. The only hypothesis in this exploratory study is a general one, that there will be some kind of interaction.

Methods

We re-analyzed an available data set from a previously reported double-blind, placebo-controlled completely counterbalanced crossover comparison of d-amphetamine and Efamol (evening primrose oil, rich in gamma-linolenic acid) as treatment for ADHD (Arnold et al. 1989). The three treatment conditions were a month long each. The subjects, 6-12-year-old boys, had zinc assays on red cells, urine, and hair by standard procedures in a university reference lab. The daily dose of d-amphetamine was a timed-release span-sule of either 10 or 15 mg, chosen according to the child's size to provide 0.35-0.7 mg/kg body weight. The Efamol dose was 4 capsules twice a day, supplying 320 mg gamma-linolenic acid (18:3n-6) per day. Conners 39-item Teacher Rating Scale and the seven-item Davids Hyperkinetic Rating Scale were collected from teachers at the end of each of the three conditions (Conners 1969; Davids 1971).

A pediatrician (S.P.) not familiar with the subjects or the data but experienced in interpreting trace mineral status blindly classified the 18 subjects as having adequate zinc nutrition ($n = 5$), borderline zinc nutrition ($n = 5$), or frank (though mild) zinc deficiency ($n = 8$), based on clinical scrutiny of red cell, hair, and urine zinc levels without seeing the subjects. No single tissue test is definitive for zinc nutrition, and the tissue of interest (CNS) was not accessible for assay. Therefore, S.P. based the classification on all three

	<i>D-amphetamine-placeo difference scores</i>		
	<i>Adequate zinc</i>	<i>Borderline</i>	<i>Zinc deficient</i>
<i>Behavioral measure</i>	<i>(n = 5)</i>	<i>(n = 5)</i>	<i>(n = 8)</i>
Conners Teacher Sum (39 items)	23.60 ± 22.68	20.00 ± 13.60	15.13 ± 28.76
Hyperactivity factor	13.20 ± 12.32	13.80 ± 9.01	10.63 ± 15.87
Hyperactivity index	10.40 ± 8.32	7.80 ± 5.22	6.38 ± 11.64
Davids Hyperkinetic Scale (sum of first 6 items by teacher)	4.00 ± 3.81	3.60 ± 3.51	1.63 ± 5.88

^aPlacebo scores minus d-amphetamine scores: means ± SD. Higher difference score is better response. The zinc deficiency (third column) is mild, picked up only by hair, red cell, and urine assays.

Table 1. Teacher-Rated Response to Dextroamphetamine by Zinc-Nutrition Categories^a

available assays, using a dynamic model of zinc balance as the net of intake/stores and excretion, with possible variations from tissue to tissue. In this model, high urine or hair zinc could be a result of either high intake or excess wasting of the normal intake, and normal red cell zinc is only suggestive of normal neuronal zinc because zinc level can vary from one tissue to another. The three tissue levels were clinically integrated thusly: If all three were high or all three normal, zinc nutrition was classified adequate. If all three were low, it was classified deficient. In the cases with "splits," hair level was first examined as the best measure of long-term zinc availability to the CNS: if it was definitely low, the classification was zinc deficiency; if hair zinc was high or within normal variability, the other tissue levels were consulted. With normal hair zinc, high or normal red cell zinc yielded a classification of adequate, and low red cell level yielded a classification of borderline. If hair zinc was high, normal red cell and urine levels or high red cell level with normal urine level yielded a classification of adequate, while normal red cell level with high or low urine level or low red cell level with normal urine level yielded a classification of borderline.

Mean placebo-Efamol and mean placebo-d-amphetamine differences from the completely counterbalanced crossover trial were then calculated for each of these three blind zinc-nutrition groupings on Conners teacher ratings (total, hyperactivity factor, and hyperactivity index) and Davids Hyperkinetic Scale teacher ratings (Tables 1 and 2). Statistical comparison tests were not done because of the small group sizes in this heuristic exploration; rather, effect size implications for possible future studies are emphasized.

	<i>Efamol-placeo difference scores</i>		
	<i>Adequate zinc</i>	<i>Borderline</i>	<i>Zinc deficient</i>
<i>Behavioral measure</i>	<i>(n = 5)</i>	<i>(n = 5)</i>	<i>(n = 8)</i>
Conners Teacher Sum (39 items)	-0.80 ± 13.41	18.60 ± 11.93	6.25 ± 19.41
Hyperactivity factor	-0.80 ± 3.42	12.00 ± 9.27	4.25 ± 8.83
Hyperactivity index	0.20 ± 2.59	6.80 ± 4.87	2.38 ± 5.93

Dauids Hyperkinetic Scale	0.00 ± 1.22	3.60 ± 2.79	0.63 ±2.13
(sum of first 6 items by teacher)			

^aPlacebo scores minus Efamol scores: means ± SD. Higher difference score is better response. The zinc deficiency (third column) is mild, picked up only by hair, red cell, and urine assays.

Table 2. Teacher-Rated Response to Gamma-Linolenic Acid (Efamol) by Zinc-Nutrition Categories^a

Results

Descriptive statistics (mean ± SD) for placebo-corrected behavioral effects of d-amphetamine for each zinc nutritional classification are shown in Table 1, and similarly for placebo-corrected effects of Efamol in Table 2. For visual comparison, the Conners teacher hyperactivity index means are graphed in Figure 1 for both the d-amphetamine-placebo and Efamol-placebo differences.

Inspection of the tables and figure suggests that d-amphetamine effect is associated linearly or perhaps asymptotically with level of zinc nutrition, while Efamol effect seems more quadratic (inverted U-shaped curve). Efamol had no effect in the five subjects with adequate baseline zinc nutrition, was practically as effective as d-amphetamine in the five subjects with borderline zinc nutrition, and faded in effect in the eight subjects with mild zinc deficiency.

The potential clinical significance is revealed by comparison of effect sizes (Cohen's d, mean difference divided by SD). An effect size of 0.3 is considered small, 0.5 moderate, and 1.0 large. For comparison, the pooled mean placebo-stimulant difference in most controlled studies has an effect size of about 0.9-1.2. In this analysis, the placebo-controlled effect size of d-amphetamine is well over 1.0 on all measures in the presence of adequate or even borderline zinc nutrition but only 0.3-0.6 (depending on the measure) in the presence of mild zinc deficiency. The placebo-controlled effect size of Efamol is nil in the presence of adequate zinc nutrition, but exceeds 1.3 on most measures in the presence of borderline zinc deficiency. In fact, both Efamol and d-amphetamine show placebo-controlled effect sizes up to 1.5 (1.2-1.6 for Efamol, 1.0-1.5 for d-amphetamine) with borderline zinc nutrition, dropping to <0.7 with mild zinc deficiency. This suggests a moderator effect size of about 0.7-0.8 for zinc deficiency compared to borderline zinc status.

Discussion

If upheld by further study, these data suggest the following: (1) zinc nutrition may be important for treatment of ADHD even by pharmacotherapy, and (2) if Efamol benefits children with ADHD, it likely does

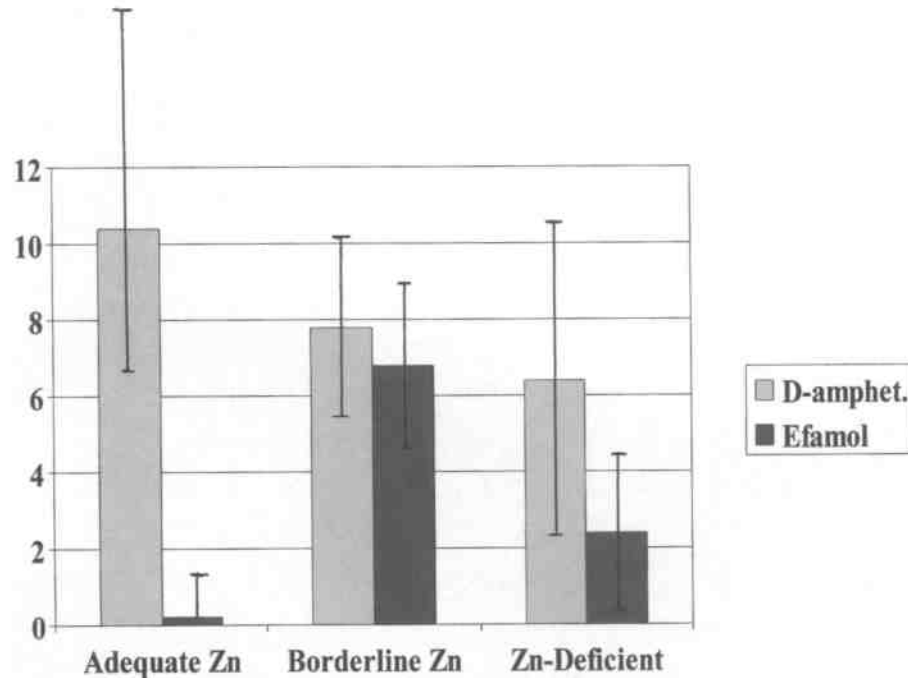


Fig. 1. Mean difference scores (\pm SE) between placebo and the two active treatments (d-amphetamine, Efamol) on teacher Connors hyperactivity index, by zinc-nutrition category determined from hair, red cell, and urine zinc assays. Placebo-controlled amphetamine benefit appears linear with zinc nutrition. Efamol benefit appears quadratic, highest with borderline zinc.

so by improving or compensating for borderline zinc nutrition; if zinc nutrition is frankly deficient (even mild deficiency), Efamol cannot make up for it. It is important to note that the children in this study were not clinically diagnosed as zinc-deficient; the deficiency was picked up only by the tissue assays.

It is not clear whether the interaction of d-amphetamine with zinc nutritional status, if upheld by further study, would also extend to other stimulants or to antidepressants. It is also not clear whether the Zn-moderated effect of Efamol, if upheld by further study, would apply to other essential fatty acid preparations, especially those with a heavy emphasis on the n-3 series. If the mechanism of any Efamol benefit is by improving absorption of borderline amounts of dietary zinc, this may be specific to the n-6 series, especially gamma-linolenic acid, the precursor of dihomo-gamma-linolenic acid, which is the precursor of the series 2 prostaglandins most implicated in gut absorption of zinc.

The proportions of this ADHD sample classified as mildly zinc-deficient (44%) and borderline in zinc nutrition (28%) deserve some comment. Though apparently high, they are not inconsistent with other published reports. As background, Sandstead (1973) found generally suboptimal zinc levels in U.S. diets. Bekaroglu et al. (1996) reported mean serum zinc of 60.6 ± 9.9 mcg/dL in 33 boys and 15 girls with ADHD compared to 105.8 ± 13.2 mcg/dL in healthy volunteers (30 boys and 15 girls). Toren et al. (1996) reported significantly lower serum zinc levels and more variance in 39 boys and four girls age 6-16 years with ADHD than in a control group of 28 age-matched healthy controls; 35% of subjects with ADHD fell outside the normal control range of 8.3-19.2 mcmol/L, most of these (30%) being lower. Rates based on a single tissue assay might be considered a lower-bound estimate of deficiency prevalence; additional tissues, as used in the study reported here, might discover more subtle deficiency and borderline states.

It is interesting to note that, though multiple studies in animals and humans support an

association of zinc nutritional state with behavior relevant to ADHD, one study that did not was from the Dunedin (New Zealand) developmental study cohort, in which McGee et al. (1990) found no significant correlation of hair or serum zinc with parent or teacher hyperactivity ratings. (This was a general epidemiologic study, not a comparison of diagnosed ADHD to normal controls.) A negative trial of Efamol for ADHD was also reported from New Zealand (Aman et al. 1987). As with selenium, iodine, and some other trace minerals, zinc intake can vary geographically. The amount of zinc in the soil and in the local flora and fauna, as well as endemic dietary habits, can influence the amount of zinc ingested. It might be illuminating to compare the New Zealand diet and its zinc content to the U.S. diet and zinc content.

The discussion above is entirely speculative at this point, of course. No definite conclusions should be drawn from these preliminary data, which are presented for their heuristic value in guiding further, hypothesis-based, prospective research.

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References

- Agget PJ, Harries JT: Current status of zinc in health and disease states. *Arch Dis Child* 54:909-917, 1979
- Alanko J, Riutta A, Vapaatalo H: Effects of catecholamines on eicosanoid synthesis with special reference to prostanoic/leukotriene ratio. *Free Radic Biol Med* 13:677-688, 1992
- Aman MG, Mitchell EA, Turbott SH: The effects of essential fatty acid supplementation by Efamol in hyperactive children. *J Abnorm Child Psychol* 15:75-90, 1987
- Arnold LE, Kleykamp D, Votolato NA, Taylor WA, Kontras SB, Tobin K: Gamma-linolenic acid for attention-deficit hyperactivity disorder: Placebo-controlled comparison to d-amphetamine. *Biol Psychiatry* 25:222-228, 1989
- Arnold LE, Votolato NA, Kleykamp D, Baker GB, Bornstein RA: Does hair zinc predict amphetamine improvement of ADHD/hyperactivity? *Int J Neurosci* 50:103-107, 1990
- Bekaroglu M, Yakup A, Yusof G, Orhan D, Hilal M, Erol E, Caner K: Relationships between serum free fatty acids and zinc and ADHD. *J Child Psychol Psychiatry* 37:225-227, 1996
- Bettger WJ, Reeves PG, Moscatelli EA, Reynolds G, O'Dell BL: Interaction of zinc and essential fatty acids in the rat. *J Nutr* 109:480-488, 1979
- Black MM: Zinc deficiency and child development. *Am J Clin Nutr* 68:464S-469S, 1998
- Chen MD, Lin PY, Sheu WH: Zinc coadministration attenuates melatonin's effect on nitric oxide production in mice. *Biol Trace Element Res* 69:269-268, 1999
- Conners CK: A teacher rating scale for use in drug studies with children. *Am J Psychiatry* 126:152-156, 1969
- Dauids A: An objective instrument for assessing hyperkinesis in children. *J Learning Disabil* 4:499-501, 1971
- Di Marzo V, Piomelli D: Participation of prostaglandin E₂ in dopamine D₂ receptor-dependent potentiation of arachi-donic acid release. *J Neurochem* 59:379-382, 1992
- Eder K, Kirchgessner M: Zinc deficiency and the desaturation of linoleic acid in rats force-fed fat-free diets. *Biol Trace Elem Res* 54:173-183, 1996
- Fogarty AC, Ford GL, Dreosti IE, Tinsley IJ: Zinc deficiency and fatty acid composition of tissue lipids. *Nutr Rep Int* 32:1009-1019, 1985
- Golub MS, Takeuchi PT, Keen CL, Hendrickx AG, Gershwin ME: Activity and attention in zinc-deprived adolescent monkeys. *Am J Clin Nutr* 64:908-915, 1996
- Gomez-Nino A, Almaraz L, Gonzalez C: Potentiation by cyclooxygenase inhibitors of the release of catecholamines

from the rabbit carotid body and its reversal by prostaglandin E₂. *Neurosci Lett* 140:1-4, 1992

Gross HA, Dunner DL, Lafleur D, Meltzer HL, Muhlbauer HL, Fieve RR: Prostaglandins: A review of neurophysiology and psychiatric implications. *Arch Gen Psychiatry* 34:1189-1196, 1977

Halas ES, Sandstead HH: Some effects of prenatal zinc deficiency on behavior of the adult rat. *Pediatr Res* 9:94-97, 1975

Huang YS, Cunnane SC, Horrobin DF, Davigon J: Interactions between zinc, essential fatty acids and prostaglandins: Relevance to acrodermatitis enteropathica, total parenteral nutrition, the glucagonoma syndrome, diabetes, anorexia nervosa, sickle cell anemia. *Med Hypotheses* 6:277-296, 1980

Kozielec T, Starobrat-Hermelin B, Kotkowiak L: Deficiency of certain trace elements in children with hyperactivity [in Polish]. *Psychiatr Pol* 28:345-353, 1994

McGee R, Williams S, Anderson J, McKenzie-Parnell JM, Silva PA: Hyperactivity and serum and hair zinc levels in 11-year-old children from the general population. *Biol Psychiatry* 28:165-168, 1990

Molderings G, Malinowska B, Schlicker E: Inhibition of noradrenaline release in the rat vena cava via prostanoid receptors of the EP3 subtype. *Br J Pharmacol* 107:352-355, 1992

Nadasz GL, Szekacs B, Juhasz I, Monos E: Pharmacological modulation of prostacyclin and thromboxane production of rat and cat venous tissue slices. *Prostaglandins* 44:339-355, 1992

Negishi M, Ito S: Prostaglandin E₂-induced arachidonic acid release and catecholamine secretion from cultured bovine adrenal chromaffin cells. *Biochem Pharmacol* 44:2315-2321, 1992

Poling JS, Vicini S, Rogawski MA, Salern N Jr: Docosahexaenoic acid block of neuronal voltage-gated K⁺ channels: Subunit selective antagonism by zinc. *Neuropharmacology* 35:969-982, 1996

Prows DR, Schroeder F: Metallothionein-IIA promoter induction alters rat intestinal fatty acid binding protein expression, fatty acid uptake, and lipid metabolism in transfected L cells. *Arch Biochem Biophys* 340:135-143, 1997

Racke K, Bahring J, Langer C, Bräutigam M, Wessler I: Prostanoids inhibit release of endogenous norepinephrine from rat isolated trachea. *Am Rev Respir Dis* 146:1182-1186, 1992

Sakuma S, Fujimoto Y, Miyata Y, Ohno M, Nishida H, Fujita T: Effects of Fe²⁺, Zn²⁺, Cu²⁺ and Se⁴⁺ on the synthesis and catabolism of prostaglandins in rabbit gastric antral mucosa. *Prostaglandins Leukot Essent Fatty Acids* 54:193-197, 1996

Sandstead HH: Zinc nutrition in the United States. *Am J Clin Nutr* 26:1251-1260, 1973

Sandstead HH, Fosmire GJ, Halas ES, Jacob RA, Strobel DA, Marks EO: Zinc deficiency: Effects on brain and behavior of rats and Rhesus monkeys. *Teratology* 16:229-234, 1997

Sandyk R: Zinc deficiency in attention/deficit/hyperactivity disorder. *Int J Neurosci* 52:239-241, 1990

Sherbourne CD, Gonzales R, Goldyne ME, Levine JD: Norepinephrine-induced increase in sympathetic neuron-derived prostaglandins is independent of neuronal release mechanisms. *Neurosci Lett* 139:188-190, 1992

Song MK, Adham NF: Evidence for an important role of prostaglandins E₂ and F₂ in the regulation of zinc transport in the rat *J Nutr* 109:2152-2159, 1980

Starobrat-Hermelin B: The effect of deficiency of selected bioelements on hyperactivity in children with certain specified mental disorders. *Ann Acad Med Stetin* 44:297-314, 1998

Toren P, Sofia E, Sela BA, Wolmner L, Weitz R, Dov I, Koren S, Reiss A, Weizman R, Laor N: Zinc deficiency in ADHD. *Biol Psychiatry* 40:1308-1310, 1996

Villet A, Ravel A, Richard MJ, Alary J, Roussel AM, Favier A: Fish oil effects on tissular fatty acids and plasma lipid peroxidation in zinc-deficient rats. *J Trace Elem Med Biol* 11:223-231, 1997

Weidenfeld J, Kabbha K, Reches A, Shohami E: Role of the central adrenergic system in the regulation of prostaglandin biosynthesis in rat brain. *J Neurochem* 58:694-699, 1992